EXHIBIT 15

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1: J Biol Chem. 1994 Jun 17;269(24):16696-700. Related Articles, Links

Mutagenesis of recombinant protein C inhibitor reactive site residues alters target proteinase specificity.

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Protein C inhibitor (PCI) is a heparin-binding plasma serine proteinase inhibitor (serpin) which is thought to be a physiological regulator of activated protein C. We are using recombinant PCI (rPCI) to study structural determinants of target proteinase specificity. A cDNA encoding full-length PCI has been expressed as a fully active proteinase inhibitor using Autographa californica nuclear polyhedrosis virus (baculovirus). rPCI was expressed maximally 4 days after infection and could be expressed either in Sf9 or High-Five cells. rPCI bound heparin and was conveniently purified with heparin-Sepharose (eluting > 0.5 M NaCl). The rPCI formed sodium dodecyl sulfate-polyacrylamide gel electrophoresis-stable complexes with thrombin and activated protein C (APC). The inhibitory properties of wild-type rPCI and plasma-derived PCI are essentially the same either in the absence or presence of heparin with thrombin, APC, trypsin, and urokinase. The residues Phe353-Arg354-Ser355 (P2-P1-P1') constitute part of the reactive site loop of PCI with the Arg-Ser peptide bond being cleaved by the proteinase. Using site-directed mutagenesis we studied the contribution of the reactive site FRS for proteinase inhibition in rPCI. Changing the P1 residue Arg354-->Met generated a reactive site similar to alpha 1-proteinase inhibitor which was a much poorer inhibitor of thrombin, APC, trypsin, and urokinase. Changing the P2 residue Phe353-->Gly generated a mutant with a reactive site like antithrombin which was better at inhibiting thrombin or urokinase, but was much less active with APC or trypsin. Changing the P1' residue Ser355-->Met generated a reactive site like plasminogen activator inhibitor-1 and this protein inhibits all the proteinases essentially like wild-type rPCI. These results show the importance of PCI's Phe353 (P2) and Arg354 (P1) in target proteinase specificity, and they further support the concept of reactive site sequences determining serpin function.